Marine Algae-Derived Bioactive Compounds

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Marine algae are rich in bioactive nutraceuticals (e.g., carbohydrates, proteins, minerals, fatty acids, antioxidants, and pigments). Biotic (e.g., plants, microorganisms) and abiotic factors (e.g., temperature, pH, salinity, light intensity) contribute to the production of primary and secondary metabolites by algae. Easy, profitable, and sustainable recovery methods include novel solid-liquid and liquid-liquid extraction techniques (e.g., supercritical, high pressure, microwave, ultrasound, enzymatic). The spectacular findings of algal-mediated synthesis of nanotheranostics has attracted further interest because of the availability of microalgae-based natural bioactive therapeutic compounds and the cost-effective commercialization of stable microalgal drugs.

Keywords: marine algae ; nanotheranostics ; bioactive compounds ; innovation ; alternative and complementary medicine ; diabetes ; neurodegenerative diseases ; marine drugs

1. Introduction

Over the last two decades, the synergy between engineering and medical science has opened novel frontiers in the field of nanotheranostics. Nanoformulation is one of the fastest developing platforms to overcome limitations in the use of peptide-based drugs [1][2]. The paramount advantages of NPs in the development of nanotheranostics have been linked to enhanced drug effectiveness in several fascinating ways, by increasing the (i) biocompatibility (safety); (ii) systemic bioavailability (half-life); (iii) solubility (drug delivery); (iv) biodistribution (sustained and controlled drug release in target tissues and cells); (v) biostability; (vi) versatility and ability to overcome sequential biological barriers (e.g., pump-mediated multidrug (MDR) resistance, sequestration by the mononuclear phagocytic system); (vii) large-scale production; (viii) simple usage (e.g., possibility of nanodrug administration by any possible route (e.g., per os, transdermal, topical, intravenous); (ix) functional efficiency (surface functionalization), while reducing the side effects (e.g., sufficiency of minimal applied nanodrug concentration) [1][2][3][4].

Different types of nanotherapeutics have been synthesized using physical, chemical, and biological methods [5][6][7][8][9][10][11][12]. Theranostic NPs are categorized into: (i) hybrid NPs (composed of different nanomaterials, such as metals, biopolymers, and lipids); (ii) multifunctional NPs (functionalized with targeting moieties and/or drugs); and (iii) multifunctional hybrid NPs (incorporating characteristics of (i) and (ii)) [7][10][12][13][14]. The most promising nanotheranostics are modifiable/functionalizable nanosystems that generally combine more than one composite with a core-shell structure [15]. Often, nanosystems are functionalized with biocompatible polymeric layers and/or targeting moieties, including contrast agents, which succeed in forming the interaction between the imaging and the therapeutic parts of NPs [10][13][15][16]. Moreover, surface functionalization of NPs allows active targeting of cells and/or a combination of light-based modalities [4][10][13][16]. Therefore, the fabrication of nanoplatforms with in vivo stability for efficient delivery of drugs or diagnostic markers to biological targets is of foremost importance, as they will be exposed to drastic conditions within the microenvironment (e.g., tumor) [14][15].

Although different NPs have been formulated as nanotheranostics, their safety concerns in humans have not been completely studied yet [4][15]. When designing nanotheranostics, the identification of major factors is crucial for clinical applications (e.g., imaging/contrast modalities, chemotherapy) [3][10][15][16][17]; this includes a deeper understanding of the mechanisms by which a given theranostic, ideally cost-effective, is (i) administered (to avoid premature release from the delivery system); (ii) cleared from the body; (iii) able to interact with the immune system; and (iv) defined as safe (e.g., by optimizing the dose levels, drug encapsulation, ligand conjugation efficiency and efficacy, drug administration frequencies, as well as by ensuring the reproducibility of the theranostics' effects in vitro, ex vivo, and in vivo (e.g., clinical trials)) [4][14][18].

It is worth mentioning that nanoformulations vary according to chemical, biochemical, and physicochemical properties of nanomaterials (e.g., particle size and surface area) [3][4][10][14][17][19]. The enhanced biological and catalytic activity, mechanical property, melting point, optical absorption, and thermal and electrical conductivity of NPs have attracted much

attention for their use as nanomedicines (e.g., in the treatment, diagnosis, monitoring, and control of biological systems) [1][10][18]. Among the different types of NPs, magnetic NPs (e.g., γ-iron(III) oxide (γ-Fe_3O_4)), noble metal NPs (MNPs) (e.g., silver (Ag), gold (Au), copper (Cu), palladium (Pd), and platinum (Pt)), as well as semiconductor NPs (e.g., cadmium sulfide (CdS), zinc oxide (ZnO), titanium oxide (TiO_2), zinc sulfite (O_3SZn), and silica/silicon dioxide (SiO_2)) are widely used as nanotheranostics in the field of drug delivery and diagnostics [3][10][20][21][22]. Physicochemical synthesis of NPs is often cumbersome and costly with the release of harmful by-products, posing a high risk to living systems [1][10][17]. Therefore, the fountainhead of nanobiotechnology is focused on the fabrication of structurally well-defined, reproducibly synthesizable NPs from biodegradable and/or biocompatible materials such as lipids, polysaccharides, proteins, or peptides [7][18][19][21].

Recently, biological synthesis of NPs using bacteria, fungi, viruses, plants, and algae has emerged as a promising field [7][19][21][23] due to (i) the use of green energy for NPs assembly, which subsequently overcomes the environmental toxicity; (ii) the large-scale synthesis; (iii) different biocompounds present in bio-organisms that help to obtain safe NPs of different morphology/shape, size distribution, composition, and stability; (iv) cost-effectiveness; and (v) versatile usage in a wide range of activities encompassing the cosmetics, theranostics, food, and textile fields.

Phyconanotechnology is becoming an exciting and upcoming area with greater scope in the synthesis of algae-based NPs [7][10][19][23]. Algae are remarkable aquatic, photosynthetic nanobiofactories, characterized by being (i) a major under-exploited reservoir of cost-effective bioactive compounds and (ii) an excellent choice to explore for applications in the renewable energy, food, pharmaceutical, nutraceutical, and cosmetic industries; with (iii) a high growth rate in sea water or controlled conditions, (iv) an ease of handling, and (v) a capacity to absorb/accumulate inorganic metallic ions; while being able to synthesize NPs in an eco-friendly, rapid, and healthier way [7][21][23][24]. Besides these overall challenges and properties, it is worth noting that not only living, but also dead algae can be used for the synthesis of nanotheranostics [7][13][17].

Algae are known to be the largest primitive photoautotrophic and polyphyletic group of eukaryotes, which perform more than 50% of photosynthesis on this planet [24]. They are classified and primarily based on their morphological features, either as microalgae (i.e., unicellular, such as diatoms, or multicellular) or as macroalgae (sometimes referred to as seaweeds). Marine algae are classified into three major distinct classes based on the presence of specific pigments. Therefore, algae can be brown (Phaeophyta/Phaeophyceae, such as *Sargassum polycystum*, *Padina pavonica*, and *Cystophora moniliformis*); blue-green (Cyanophyta/Cyanophyceae, such as *Spirulina platensis*, *Chlorococcum humicola*, and *Chlorella vulgaris*); green (Chlorophyta/Chlorophyceae, such as *Chlamydomonas reinhardtii*, *Ulva fasciata*, and *Gracilaria edulis*); or red (Rhodophyta/Rhodophyceae, such as *Palmaria decipiens*, *Gelidiella acerosa*, and *Gracilaria corticata*) [3][7][19][21][23][25][26][27][28][29][30][31][32].

Marine algae are rich in bioactive compounds such as carbohydrates, proteins, minerals, polyunsaturated fatty acids (PUFAs), fatty acids (FAs), amines, amides, antioxidants (e.g., polyphenols, tocopherols), and pigments such as carotenoids, chlorophylls, carotene, xanthophylls, and phycobilins (phycocyanin (PC), phycoerythrin (PE)), which serve as stabilizing/capping and reducing agents for the synthesis of thermodynamically stable NPs [3][7][21][24][27][28]. It is important to mention that the virtue of biological moieties is influenced by (i) biotic (i.e., type of algal species) and (ii) abiotic factors (e.g., nutrient availability (e.g., nitrogen (N), phosphorous (P), potassium (K)), temperature, pH, salinity, inorganic carbon (C), oxygen (O_2), light intensity, and carbon dioxide (CO_2)), as well as by (iii) the dynamic algal-associated halobionts [3][21][24][28]. Most of these factors can have an impact on the production of algae and their metabolites (e.g., primary and secondary), which may subsequently affect the stability, size, and shape of NPs [7][21][24][33][34]. However, for the complete incorporation of these biomolecules in NPs—as food preservatives, prebiotics, antibiofilms, antifouling, antibiotics, or coating (in active packaging)—it is essential to achieve easy, profitable, and sustainable recovery methods [7][24][31][35]. To achieve this purpose, novel solid-liquid, and liquid-liquid extraction techniques (e.g., supercritical, high pressure, microwave, ultrasound, enzymatic, accelerated solvent, and intensity pulsed electric fields extraction) have been studied [7][33][35][36].

NPs synthesized using algae are of two types, (i) organic NPs (e.g., poly-ε-lysine, chitosan, cationic quaternary polyelectrolytes, and quaternary ammonium compounds), and (ii) inorganic/metallic NPs (e.g., Ag, Au, Pt, Pd, Cu, ZnO, TiO_2, γ-Fe_3O_4, and CdS) [3][7][15][21][23]. They are synthesized using two routes of biological extracts, namely (i) extracellular (i.e., NPs synthesized outside the cell, mainly supported by the exudates of cell metabolism comprising metabolites, ions, pigments, lipids, microbial by-products such as hormones and antioxidants, various enzymes, and non-protein entities such as DNA and RNA) and/or (ii) intracellular (i.e., NPs synthesized inside the cell, mainly supported by NADPH or NADPH-dependent reductase originated in metabolic pathways such as photosynthesis, respiration, and

nitrogen fixation) [7][15][21][23]. The synthesis of NPs was initially intracellular before its switch to the extracellular mode of synthesis [7].

Among all algal-mediated NPs, metallic NPs (MNPs) are more potent due to their unique optical and electronic properties and biocompatible nature, which has increased their usage in the biomedical field [7][21][23][37]. Their inert nature, low toxicity, and small size (increasing their cell penetration) make them potent candidates for safer and targeted theranostic applications (e.g., drug/gene delivery, gene delivery, immunoassays, tissue repair, laser-assisted therapy (e.g., photodynamic therapy (PDT) and photothermal therapy (PTT)), and/or imaging modalities (e.g., magnetic resonance imaging [MRI], positron emission tomography (PET), biosensing, and cancer chemotherapy)) [1][7][15][21][25].

2. Algal-Sourced Compounds of Medical Interest

Over the past few decades, marine algae have attracted much interest as potentially renewable resources. There are approximately 8000 different classes of species of marine algae that have been identified in the world [24][27][38][39]. Seaweeds are an excellent source of primary metabolites (e.g., polysaccharides, proteins, amino acids, dietary fiber, essential FAs) and secondary metabolites (e.g., pigments, phytosterols, polyphenols, terpenoids, carotenoids, tocopherols, minerals, and vitamins), which are known to exert cytostatic, anti-viral, anti-helminthic, anti-fungal, and anti-bacterial activities [7][33][35][40][41][42].

Based on the mechanistic differences, physiologically active substances present in marine algae are classified into two types [38][43]: (i) non-absorbed high-molecular materials and (ii) absorbed low-molecular materials, which affect the maintenance of human homeostasis directly. Currently, algal substances are used in fresh and processed foods and have gained importance in nutritional sciences, with promising pharmacological applications as antioxidant, anti-inflammatory, anti-proliferative, anti-thrombotic, anti-coagulant, anti-hypertensive, anti-diabetic, and cardio-protection properties [35][42][44]. Edible algae are therefore used as a food additive/supplement all around the world in the preparation of salads, soups, and low-calorie foods, and represent a regular meal in Japan, Korea, the USA, France, and Chile [38][39][40]. Lately, many clinically viable and commercially available novel drugs with antitumor, anti-infective, anti-diabetic, and neuroprotective formulations from macroalgal biocompounds have emerged as a rising pharmacological field [15][38][42][45][46][47]. Moreover, seaweed biocompounds have revealed their anti-diabetic and neuroprotective effects through various research studies aiming at the prevention of diabetic and neurological disorders (neurodegeneration) and the reduction of oxidative stress in the central nervous system (CNS). The field of seaweed-based anti-diabetic and neuroprotective compounds, however, is still in its infancy, requiring further discoveries and investigations.

2.1. Fatty Acid Content

Lipids in seaweeds are present in relatively low contents (i.e., 1–5% of dry weight), and these lipids consist of essential FAs and functional lipid fractions such as PUFAs (i.e., 25% and 60% of total lipids), phytosterols, glycolipids, phospholipids, and fat-soluble vitamins (carotenoids, vitamin A, D, E, and K) [24][48][49].

The most predominant PUFAs occur in the form of omega-3 (eicosapentaenoic acid (EPA; C20:5n−3), docosahexanoic acid (DHA; C22:6n−3), stearidonic acid (SDA; C18:4n−3), α-linolenic acid (LA; C18:3n−3)) and omega-6 (arachidonic acid (AA; C20:4n−6), α-linoleic acid (ALA; C18:2n−6), γ-linoleic (GLA; C18:3n−6)) [24][48]. Essential FAs are nutraceuticals added to dietary supplements or consumed as part of a balanced diet [33]. In 2004, the Food and Drug Administration (FDA) claimed that food containing PUFA omega-3 compounds are pharmacologically important, providing multiple health benefits through their ability to (i) regulate membrane fluidity, blood pressure, and blood clotting; (ii) reduce the risk of cardiovascular diseases (CVD), osteoporosis, and diabetes; and (iii) correct the development and functioning of the brain and nervous system [50][51]. Marine algae such as *Isochrysis galbana*, *Ulva fasciata*, *Laurencia papillosa*, *Gracilaria salicornia*, *Dictyota fasciola*, *Taonia atomaria*, *Chaetoceros*, *Tetraselmis*, *Thalassiosira*, and *Nannochloropsis* are known to produce high amount of PUFAs (ALA, GLA, LA, SDA, AA, and EPA) [15][48]. Furthermore, Peng et al. claimed that green seaweeds like *Ulva pertusa* predominantly contain hexadecatetraenoic, oleic, and palmitic acids [51][52]. EPA, DHA, monounsaturated FAs (C12:1 (lauroleic acid), C14:1 (myristoleic acid), C16:1 (palmitoleic acid), C17:1 (cis-10-heptadecenoic acid), and C18:1 (oleic acid)) are dominant in *Undaria pinnatifida* [48].

More than 200 types of phytosterols (662–2320 mg/g dry weight) have been found in marine algae. Brown algae such as *Agarum cribosum, Undaria pinnatifida*, and *Laminaria japonica* contain major phytosterols derivatives (e.g., fucosterol, which represents 83–97% of the total phytosterol content) [53][54][55][56].

Phospholipids in seaweed vary between 10 and 20% of the total lipids, are more resistant to oxidation (rancidity), and display a high amount of FAs, such as EPA and DHA [40][57].

Glycolipids are present in more than 50% of algal content and are characterized by high n−3 PUFAs compounds (e.g., monogalactosyldiacylglycerides, digalactosyldiacylglycerides, and sulfoquinovosyldiacylglycerides) [33].

Carotenoids are diverse and widespread lipophilic colored compounds in nature, consisting of astaxanthin, β-carotene, lutein, lycopene, and canthaxanthin [13][40]. Moreover, these characteristics give algal lipids better bioavailability and a spectrum of health benefits for humans and animals [15].

2.2. Protein Content

Proteins are biological macromolecules present in algae in single (amino acids) or conjugated (heteroproteins such as phycobiliproteins and glycoproteins) forms, and represent 20% and 67%, respectively [57][58]. The highest protein content (i.e., 10–47% of dry weight) was found in edible green (e.g., *Caulerpa lentillifera*) and red seaweeds (e.g., *Eucheuma cottonii*), compared to brown seaweeds (5–24%) (e.g., *Sargassum polycystum*) [51][57][58]. Mohamed et al. reported that most seaweed proteins contain all the essential amino acids at levels close to that recommended by Food and Agriculture Organization (FAO)/World Health Organization (WHO) [59]. Moreover, *Rhizoclonium riparium*, *Enteromorpha intestinalis*, *Lola capillaris*, *Ulva lactuca*, *Dictyota caylinica*, *Catenella repens*, *Polysiphonia mollis*, *Gelidiella acerosa*, *Capsosiphon fulvescens*, *Ulva prolifera*, *Porphyra* sp., *Osmundea pinnatifida*, *Pterocladium capillacea*, *Sphaerococcus coronopifolius*, *Gelidium microdon*, *Cystoseira abies-marina*, *Fucus spiralis*, and *Ulva compressa* have significant levels of proteins [57][58][59]. Proteins display anti-inflammatory, antioxidant, anti-tumor, anti-aging, and protective activity and are therefore beneficial for the prevention and treatment of neurodegenerative diseases, cancers, gastric ulcers, DNA replication, response to stimuli, transport of molecules, and catalysis of biochemical reactions [40][59][60].

Besides, amino acids are applied as natural moisturizing agents to hair and skin, and are therefore beneficial in functional pharmaceuticals, nutraceuticals, and cosmeceuticals [31]. Macroalgal species like *Chlorella* sp., *Dunaliella salina*, *Aphanizomenon flos-aquae*, *Dunaliella tertiolecta,* and *Spirulina plantensis* are widely used as human food sources because of their rich protein content and high nutritive value [42]. Some species of algae are good sources of endogenous (e.g., glutamic acid, aspartic acid, threonine, proline, serine, and glycine) and exogenous (e.g., phenylalanine, histidine, isoleucine, leucine, lysine, methionine, threonine, tryptophan, and valine) amino acids [57][60]. *Ulva australis* contains histidine and taurine, *Ulva* spp. contains aspartic and glutamic acid (26–32% of the total amino acid), and *Palmaria palmata* (Dulse) and *Himanthalia elongata* (sea spaghetti) contain high concentrations of serine, alanine, and glutamic acid, while *Sargassum vulgare* contains a high level of methionine [58][59]. Moreover, mycosporine-like amino acids (MAAs) have been detected in diverse organisms and especially in Rhodophyta; *Chondrus crispus*, *Palmaria palmata*, *Gelidium* spp., *Porphyra/Pyropia* spp., *Gracilaria cornea*, *Asparagopsis armata*, *Solieria chordalis*, *Grateloupia lanceola*, and *Curdiea racovitzae* [60]. MAAs, produced directly or indirectly in algae, can absorb solar energy, and protect marine organisms when exposed to high ultraviolet (UV) radiation [31][61]. Besides, these algal species can be potentially used in cosmetics and toiletries as activators of cell proliferation and UV protectors [31][60][61].

Phycobiliproteins are composed of a protein covalently linked to chromophores called phycobilins (i.e., PC and PE) [57][58]. These water-soluble proteins are good antioxidants and can be used as a natural food colorant [15]. PC, a blue-colored phycobiliprotein produced essentially from the cyanobacteria *Arthrospira* spp., and PE (pink-colored protein pigment) produced by the cyanobacteria *Lyngbya* spp. showed anticancer properties against A549 lung cancer cells [33][40].

Glycoproteins are another type of protein present in marine algae that consist of proteins bound to carbohydrates. About 36.24% of the glycoproteins consist of rhamnose, galactose, glucose, and mannose, with a mole ratio of 38:30:26:6 [15][60].

2.3. Carbohydrate Content

Polysaccharides represent 76% of the algal dry weight [15]. These are the major constituents in the cell wall structure of algae, and play important physiological functions [40][41][57]. The algal polysaccharides (e.g., fucan, fucoidans, galactan sulfate, carrageenans, xylomannan sulphate, sodium alginate, fucoxanthin, porphyrin, and alginic acid) found in the cell wall vary with the algae genera and species and can be broadly grouped into sulfated and non-sulfated [40][62][63].

Different amounts of sulfated polysaccharides are found in Chlorophyta (e.g., fucoidans, agar, ulvans, and carrageenans), Phaeophyta (e.g., laminaran, alginate, and fucan), and Rhodophyta (e.g., agar and carrageenans) [41][63]. Minor sulfated polysaccharides such as fucoidans, xylans, and ulvans are found in brown, red, and green seaweeds, respectively [35].

Sulfated polysaccharides extracted from the intercellular space and the fibrillar wall of green seaweeds account for 9 to 36% of algal dry mass in *Ulva* spp. [57]. *Chlorella ellipsoidea* showed several health benefits, such as the capacity to lower blood sugar levels, increase hemoglobin concentration, and act as hepatoprotective and hypocholesterolemic agents.

Several food products such as powdered green tea, soups, noodles, bread and rolls, cookies, ice cream, and soy sauce now have been developed with the use of *Chlorella* sp., in which the most important substance is β-1,3-glucan, which is an active immunostimulator, a free radical scavenger, and a reducer of blood lipids [35][60].

Carrageenans are major polysaccharides of the red algal cell wall, and consist of three general forms classified according to the degree of sulphation: kappa, lambda, and iota [33]. Carrageenans, as well as galactan and xylomannan sulphates found in red seaweeds, exert good antiviral properties on the formation of formally similar complexes that block the interaction of the viruses with the cells [64]. Carrageenans obtained from *Hypnea* spp. (but also from the green alga *Ulva lactuca*) exhibit antiviral and antioxidant properties and significant hypocholesterolemic activities by reducing cholesterol and sodium absorption while enhancing potassium absorption [57].

Agar is a mixture of two polysaccharides, namely agarose and agaropectin, which are also extracted from red seaweeds found to have similar structural and functional properties as carrageenans [33][63].

Porphyran, a complex sulfated polysaccharide obtained from the red *Porphyra* spp., has been found to exert immunoregulatory, antioxidant, and antitumor activities [35][41][59].

The sulfated polysaccharides like glucuronic acid, galactose, glucose, rhamnose, and arabinose isolated from the microalgae *Spirulina platensis* exhibited antiviral activity, and those isolated from the red algae *Gracilariopsis lemaneiformis* (i.e., 3,6-anhydro-l-galactose and d-galactose) showed high activity against A549 lung cancer cell line [35][50].

Fucoidans polysaccharides, used to develop novel medicines and functional foods, are generally produced by brown algae such as *Sargassum thunbergi*, *Ascophyllum nodosum*, *Viz fucusvesiculosus*, *Laminaria japonica*, *Fucus evanescens*, and *Laminaria cichorioides* [57]. Algae fucoidans possess antioxidant, antiproliferative, antitumor, antiviral, anti-inflammatory, anti-coagulant, anti-peptic, antiadhesive, antithrombotic properties. They also exhibit high anticancer activity against lung cancer and can suppress lung cancer metastasis by inhibiting matrix metalloproteinases (MMPs) and Vascular Endothelial Growth Factor (VEGF) [40][59]. Fucoidans can present a synergistic effect towards the anticancer agents currently in use [63]. Thus, these polysaccharides can be incorporated into or combined with existing conventional medicines to improve their efficacy. Soluble dietary fibers obtained from *Eucheuma cottonii*, *Caulerpa lentilifera*, *Sargassum polycystum*, *Ahnfeltiopsis concinna*, *Gayralia oxysperma*, *Sargassum obtusifolium*, *Chondrus ocellatus*, and *Ulva fasciata* were shown to reduce blood cholesterol levels and deter metabolic syndrome [40][57].

Alginate (β-d-mannuronic acid, α-l-guluronic acid, d-guluronic, and d-mannuronic) is a commercially available (in acid and salt forms) non-sulfated polysaccharide extracted from the dark brown seaweed *Laminaria digitata* [33][63]. The literature has shown that alginates extracted from brown seaweeds possess a higher nutritional role, and are potentially beneficial in gut health, contributing to water binding, fecal bulking, and decrease of colon transit time, which is a positive factor in preventing colon cancer [41][65]. Moreover, alginates affect the bioabsorption of minerals due to their binding nature, help to maintain body weight and deter overweight and obesity, and reduce hypertension [33][41].

2.4. Mineral Content

Seaweeds contain significant amounts of essential minerals, including macroelements (e.g., Na, P, K, calcium (Ca), and magnesium (Mg)) and trace elements (e.g., iron (Fe), zinc (Zn), manganese (Mn), and Cu), due to their marine habitat [38][60]. For instance, the green algae *Ulva clathrata* in México contains a total mineral content of 49.6% of dry matter [57].

Minerals, along with cell surface polysaccharides (e.g., agar, carrageenans, alginic acid, alginate, and cellulose), play an important role in building human tissues and regulating vital reactions as cofactors of many metalloenzymes [40][60]. Hence, seaweeds are an important source of minerals, and are regarded as beneficial functional foods (i.e., food supplements) after daily intake [42]. It is important to mention that the mineral content in brown algae is higher than in red algae [38].

Most edible seaweeds contain relatively higher Na and Ca concentration levels compared to that of terrestrial foods (e.g., apples, oranges, carrots, and potatoes). Intake of low Na:K ratios helps to reduce the incidence of hypertension, and algae usually contain Na:K ratios below 1:5 [57][60]. Besides, minerals like Fe and Cu are present in seaweeds at higher concentration levels than in meats and spinach [57]. Moreover, Cu, iodine (I), Mg, Zn, and Fe are abundant in seaweeds. Iodine is an antioxidant, anti-goiter, anticancer agent, and an important nutrient in metabolic regulation found in several forms (e.g., I^-, I_2, IO_2^-). However, consumption of very large amounts of I could induce some undesirable effects [57][59].

Arsenic (As) is among the trace elements present in algae that can display poisonous health effects [57]. Nevertheless, further analysis of speciation indicates that the type of As is important in assessing toxicity, and the levels of heavy metals remain normally below food safety limits in most marine algae [48].

Therefore, edible seaweeds could be used as a regular food or as a food supplement to help meet the recommended daily intake of some macrominerals and trace elements [48][60].

2.5. Vitamin Content

Vitamins are organic compounds that contribute to essential micronutrients in many biological activities as coenzymes or precursors (e.g., vitamins B6/pyridoxine, B12/cobalamin, and B9/folic acid) and as a part of the antioxidative defense system (e.g., vitamin C/ascorbic acid, carotenoid, and vitamin E/tocopherol) [51][57].

Seaweeds are excellent sources of water (B1/thiamine, B2/riboflavin, B3/niacin, B5/pantothenic acid, B6, B9, B12, C, H/biotin) and fat-soluble vitamins (A/retinoic acid, D, E (which includes α-tocopherol (5,7,8-trimethyltocol), β-tocopherol (5,8-dimethyltocol), γ-tocopherol (7,8-dimethyltocol), and δ-tocopherol (8-methyltocol)), and K) with antioxidant properties [38][48][57][60].

Studies suggest that eating *Spirulina*, which is rich in provitamin A and vitamin B12, increases *Lactobacillus* spp. in the gut and facilitates more efficient absorption of vitamin B1, among many others [38].

Water-soluble vitamins, such as vitamin C, are present in large amounts in *Ulva lactuca*, *Eucheuma cottonii*, *Caulerpa lentillifera*, *Sargassum polycstum*, and *Gracilaria* spp., and help in inhibiting low-density lipoproteins (LDL) oxidation and the formation of thrombosis/atherosclerosis [57]. A relatively high level of dried β-carotene (e.g., 197.9 mg/g in *Codium fragile* and 113.7 mg/g in *Gracilaria chilensis* sp.) was found in red algae compared to other vegetables (e.g., 17.4 mg/g in *Macrocystis pyrifera*) [42][48], while brown seaweeds (e.g., *Undaria pinnatifida*) contain higher levels of a-tocopherol/vitamin E (99% of the total vitamins) compared to green and red seaweeds [60].

The main fat-soluble vitamins (A and E) increase the production of nitric oxide (NO) and nitric oxide synthase (NOS) activity, thereby helping to prevent CVDs [51][57]. Besides, vitamin E exerts an antioxidant activity, which is capable of inhibiting the oxidation of LDL [40].

2.6. Pigments

Natural pigments are important for photosynthetic algae metabolism, and based on their pigment contents, macroalgae are classified into three basic groups: Chlorophyceae (green algae), Phaeophyceae (brown algae), and Rhodophyceae (red algae) [33][38][40][51]. Macroalgae can synthesize three basic classes of natural pigments: (i) chlorophylls, (ii) carotenoids, and (iii) phycobilins [38][50]. Macroalgae rich in chlorophylls a and b appear green, while the greenish-brown color of algae is attributed to the presence of fucoxanthin (carotenoid) and the red color of algae is due to the presence of chlorophylls a, c, and d and phycobilins (i.e., PE and PC) [33][51][60][66].

Chlorophylls are greenish lipid-soluble natural pigments that contain a porphyrin ring. These can be divided into four groups: chlorophyll a, chlorophyll b, chlorophyll c, and chlorophyll d [51].

Carotenoids have recently gained interest and are used for dietary supplements, fortified foods, food dyes, animal feed, pharmaceuticals, and cosmetic products due to their antioxidant properties that help reduce the risk of CVDs, cancers, and ophthalmologic diseases [50]. Carotenoids are lipophilic, linear polyenes, and are usually divided into two classes, which are (i) carotenes (α-, γ-, β-) and lycopenes (when the chain ends with a cyclic group, containing only carbon and hydrogen atoms) and (ii) xanthophylls (e.g., fucoxanthin, violaxanthin, antheraxanthin, zeaxanthin, lutein, neoxanthin) or oxycarotenoids (which have at least one oxygen atom as a hydroxyl group, as an oxy-group, or as a combination of both) [33][38][40]. It has been found that α- and β-carotene, lutein, and zeaxanthin are present in red seaweed; β-carotene, lutein, violaxanthin, neoxanthin, and zeaxanthin are found in green seaweed species; and β-carotene, violaxanthin, pheophytins, and fucoxanthin are found in brown algae [33][38][59]. Fucoxanthin, which belongs to the class of xanthophylls and non-provitamin A carotenoids, is found in *Alaria crassifolia*, *Ascophyllum nodosum*, *Chaetoseros* sp., *Cladosiphon okamuranus*, *Cylindrotheca closterium*, *Cystoseira hakodatensis*, *Ecklonia stolonifera*, *Eisenia bicyclis*, *Fucus serratus*, *Hijikia fusiformis*, *Himanthalia elongata*, *Ishige okamurae*, and *Fucus vesiculosus.* It is more effective against Gram-positive (e.g., *Staphylococcus aureus*, *Streptococcus agalactiae*, *Staphylococcus epidermidis*, *pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *and Serratia marcescens)* and Gram-negative (e.g., *Acinetobacter lwoffii*, *Escherichia coli*, *Klebsiella oxytoca*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *and Serratia marcescens*) bacteria [51][53][67].

Phycobiliproteins are water-soluble and natural fluorescent proteins that can be divided into three types: (i) PC (blue pigment), (ii) PE (red pigment), and (iii) allophycocyanins (light-blue pigment), with PE being the most abundant in many red macroalgae species [40][50][66]. Algae such as *Spirulina*, *Botryococcus*, *Chlorella*, *Dunaliella*, *Haematococcus*, and *Nostoc* have been recognized as great sources of phycobiliproteins. A recent study has evaluated that these pigments possess antioxidant, anti-carcinogenic, anti-inflammatory, anti-obesity, anti-angiogenic, and neuroprotective activities [40].

2.7. Polyphenols

Polyphenolic compounds are secondary metabolites (i.e., not directly involved in primary processes such as photosynthesis, cell division, or reproduction) of algae, and are characterized by an aromatic ring with one or more hydroxyl groups [33][38][57].

Polyphenols are divided into two groups, called phloroglucinols and phlorotannins. Phloroglucinols contain an aromatic phenyl ring with three hydroxyl groups, while phlorotannins are oligomers or polymers of phloroglucinol with additional halogen or hydroxyl groups [40][51][57]. Phlorotannins can be further subdivided into six groups: (i) phlorethols (aryl-ether linkage); (ii) fucols (aryl-aryl bonds); (iii) fucophlorethols (ether or phenyl linkage); (iv) eckols (dibenzo [1][4] dioxin linkages); (v) fuhalols (ortho-/para-arranged ether bridges containing an additional hydroxyl on one unit); and (vi) carmalols (dibenzodioxin moiety) [33][40].

Green and red algae contain high proportions of bromophenols, phenolic acids, an flavonoids, while brown algae predominantly contain phlorotannins (including bromo-, chloro-, and iodo-) [38]. Several reports have evaluated the effective antibacterial effect of phlorotannins, including from *Ecklonia kurome*, against several food-borne pathogenic bacteria (e.g., methicillin-resistant *Staphylococcus aureus* (MRSA) strains, *Campylobacter* spp., and *Streptococcus pyogenes*) [33][57][60].

3. Qualitative and Quantitative Aspects of Algal-Derived Biocompounds

Phytochemical profiling of algal samples by advanced analytical techniques revealed the presence and relative amounts of different phytochemicals, many with important medicinal properties (e.g., antimicrobial, anti-inflammatory, antioxidant) [8][24]. Preliminary qualitative phytochemical analysis was carried out to identify the secondary metabolites such as alkaloids, flavonoids, terpenoids, steroids, tannins, phenols, quinones, glycosides, flavanones, flavonols, steroids, and saponins present in the alcoholic/aqueous extracts of marine algae [68][69][70]. The variation in the antimicrobial and antioxidant activities were due to various parameters at the time the algal samples were collected. These parameters include the (i) presence and relative number of secondary metabolites (of phenolic or free hydroxyl nature) in algae, (ii) method of extraction of the biocompounds and the solvent used in this extraction, (iv) maturity stage of algae, and (v) environmental conditions (e.g., habitats, seasons) [70][71][72].

Qualitative colorimetric methods were used to evaluate the phytocompounds, and among the different procedures, methanolic extracts were found to have the highest reducing power in comparison with other solvents, such as ethanol, chloroform, and acetone [70][73]. However, results remain controversial among different studies and seem to be species-specific [70]. The maximum content of phenolic compounds, such as tannins and flavonoids, has been found in red and brown seaweeds [68]. Hasan et al. showed that *Hypnea musciformis* and *Enteromorpha intestinalis* algae collected from the Bay of Bengal possessed high contents of polyphenols associated with high potential of antimicrobial activity [69].

Other phytochemical screenings of different algal extracts were assessed using standard methods. An $FeCl_3$ test for tannins in methanolic extracts was assessed for brown seaweeds (i.e., *Dictyota dichotoma* and *Sargassum wightii*), green seaweeds (i.e., *Cladophora glomerata*, *Ulva lactuca*, and *Ulva reticulata)*, and red seaweeds (i.e., *Jania rubens*, *Corallina mediterranea*, and *Pterocladia capillacea*), and the results revealed that tannins are common phytocompounds in seaweeds [68][70]. These algal species can be used as a drug for gonorrhea and as healing agents, and seem to exert anti-viral, anti-bacterial, and anti-ulcer activities [50][65]. A Mayer test was used to qualitatively identify the contents of alkaloids in *Dictyota dichotoma*, *Jania rubens*, *Cystoseira mediterranea*, and *Pterocladiella capillacea* [68]. These are important as antimicrobial agents to inhibit the growth of both Gram-positive and Gram-negative bacteria [70]. Flavonoids, flavonols, quinones and glycosides, flavanones, saponins, and steroids were evaluated qualitatively using the Shinoda test, NaOH test, foam test, and Liebermann–Burchard test, respectively, in different algal species to analyze their therapeutic values [73][74]. In addition, an NaOH test was employed to detect the higher quantity of coumarins in Rhodophyta species (i.e., *Gracilaria salicornia* and *Mastophora rosea*), which, because of their peculiar physicochemical features, were found to display an anticoagulant activity to treat lymphedema [75]. Moreover, saponins and steroids were analyzed through this method in Chlorophyta species (i.e., *Halimeda cuneata* and *Pseudocodium devriesii*) and Phaeophyta (i.e., *Pelvetia wrightii* and *Dictyota dichotoma*) [68][70].

Quantitative analysis of flavonoids, tannins, and phenolics are usually carried out using aluminum chloride assay, 2,2-azinobis 3-ethylbenzothiazoline-6-sulfonate (ABTS) radical scavenging assay, hydroxyl radical scavenging assay, Fe^{2+} chelation assay, and Folin–Ciocalteu reagent (FCR) methods [69][73][76].

As evoked earlier, marine algae also possess a range of macro- and micro-elements required by humans and animals, such as Ca, Na, Mg, K, P, I, Fe, and Zn [72][77]. Semiquantitative and discriminant analyses were used to calculate different percentages of such elements (e.g., Ca, Mg, Na, and K), even within the same group of seaweeds, to differentiate the type of seaweed according to their quantitative mineral levels [77]. For instance, K is known to be present in high proportions in some Phaeophyta species (e.g., *Padina arborescens*, *Hizikia fusiforme*, and *Sargassum thunbergia*), while Ca was in high proportion in other Phaeophyta species (e.g., *Scytosiphon lomentaria* and *Sargassum tortile*). In addition, Mg was found in relatively high quantities in Chlorophyta (e.g., *Ulva conglobata*, *Ulva pertusa*, and *Enteromorpha compressa*), and Chlorine (Cl) was predominantly found in *Pseudocodium devriesii*, *Gracilaria Salicornia*, and *Mastophora rosea* [72][77][78].

Each algal extract obtained is generally mixed with impurities and consists of one or multiple components; therefore, analysis using separation techniques is very important [74]. Different analytical techniques such as high-performance liquid chromatography (HPLC), gas chromatography (GC), thin-layer chromatography (TLC), mass spectrometry (MS), nuclear magnetic resonance (NMR), and one or more combined techniques, such as high performance liquid chromatography–mass spectrometer (HPLC–MS), gas chromatography–mass spectrometry (GC–MS), and high performance liquid chromatography–diode array detection (HPLC–DAD) were used for the identification of bioactive compounds from algal extracts [8][35][36][63][79][80].

Carotenoids and chlorophylls are the most exploited fraction of algae pigments. Due to the lipid peroxidation ability of carotenoids in tissues, in-vivo studies of different biomass extracts were important [65][80]. Furthermore, the total antioxidant activity of carotenoid extracts has been evaluated by UV–Visible (UV–Vis) spectrophotometric methods and/or enzymatic assays [36]. In addition, carotenoids and chlorophylls were quantified by HPLC–photodiode array (HPLC–PDA), identifying all-trans-zeaxanthin, all-trans-lutein, all-trans-β-carotene, all-trans-α-carotene, chlorophyll-α, chlorophyll-β, pheophytin-α, and hydroxychlorophyll-α in the green microalgae *Chlorella sorokiniana* and *Scenedesmus bijuga* [36][80]. Furthermore, HPLC-PDA-MS/MS, HPLC equipped with UV detectors, and MS/MS were used for identification and/or quantification of the carotenoids from algal biomass spectrometry [19][79][81]. Liquid chromatography–mass spectrometry (LC–MS) coupled with PDA and MS showed a high sensitivity for carotenoids and carotenoid esters detection [19]. To investigate antioxidant and anti-cancer properties, the analysis of carotenoids (e.g., β-carotene) has been performed by HPLC–UV/Vis or HPLC–DAD [79][81]. Moreover, for liquid-liquid extracts (analysis done by dissolving the dry extract in the compatible solvents) and the identification of compounds (e.g., astaxanthin, canthaxanthin), HPLC–DAD represents a powerful technique [51].

HPLC is the most sensitive method and is extensively used to separately identify a wide range of compounds like flavonoids and lipids [10][36][81]. Thus, to obtain an adequate measure of the antioxidant potential of individual molecules, pre-column reaction with 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical with ultra-HPLC (UHPLC) separation was used [62][82][83]. Thereby, isoflavonoids, a class of flavonoids, can be structurally distinguished from other flavonoids using HPLC. Isoflavonoids present in brown (e.g., *Undaria pinnatifida, Sargassum muticum*, and *Sargassum vulgare*) and red (e.g., *Hypnea spinella, Halopytis incurvus, Chondrus crispus*, and *Porphyra* sp.) seaweed species were analyzed using modified methodologies of UHPLC–MS/MS [73][81]. In addition, the DPPH free radical scavenging method in cooperation with UHPLC–PDA analysis revealed the presence of two radical scavenging xanthophyll fragments, namely diadinoxanthin and diatoxanthin [84]. Furthermore, HPLC was found to be an alternative method for lipid analysis because it can potentially resolve all the various classes of lipids in crude lipid extracts [81]. Furthermore, HPLC–MS can be used to obtain a more detailed picture of lipid species within each class [79][81]. When using HPLC, sample pretreatment is important; therefore, methanol was used for dissolving the residue, while fat-soluble impurities were extracted with hexane [85]. In some cases, normal phase HPLC coupled in parallel to an evaporative light-scattering detector (ESLD) and quadrupole MS was used to detect a large amount of saturated hydrocarbon in crude lipid extracts [19][81].

In most cases, especially for analytical research and the development of nutraceuticals, it is necessary to evaluate the suitability of the analytical techniques. Algal lipid quantification is generally carried out based on indirect methods, such as Nile red fluorescence or related dye-partition assays, gravimetric measurement of crude lipid extracts, or GC analysis of lipid-derived fatty acid methyl ester (FAME) [51][81][85]. Numerous anomalies can affect neutral lipid quantification, including distortions due to β-carotene, complex kinetics of the fluorescent signal, and issues with sensitivity or specificity. Nile red fluorescence is visibly specific for lipid droplets, and is used as one of the most popular methods of algal lipid analysis [81].

GC/MS and NMR techniques are also used for lipid analysis [81]. GC is a popular method used on its own and/or in combination with various detection techniques such as PDA, UV, MS, MS/MS, HPLC, electron capture detector (ECD), and flame ionization detector (FID) [79]. With GC analysis, acyl constituents and FAME, derived from both neutral and polar lipids, can be selectively analyzed in each lipid extract [85]. Algal-derived FAs, as methyl or ethyl esters, could be then analyzed by LC–MS and/or GC–FID [85]. Moreover, post-methylated lipid analyses can be carried out using GC–MS. Reversed-Phase HPLC (RP–HPLC) was a widely applied analysis method, but this technique fails to separate highly polar compounds from the less polar ones [36]. Therefore, capillary electrophoresis (CE) using DAD (CE–DAD), which shows shorter application time, higher efficiency, and selectivity, is used as a substitute method to RP–HPLC for fast SFE extracts characterization [36].

NMR, MS, HPLC–MS, HPLC–UV–MS, and GC–MS have been applied to perform a pharmaceutical-grade analysis of biocompounds. For terpenes, GC–MS or NMR were found to be applied for structural determination. GC coupled to an electrospray ionization (GC–ESI) and GC–MS analyses are very selective for identification of heat-labile components (e.g., volatile materials, hydrocarbons, and FAs) in phytoextracts [36][79]. 1D- and 2D-NMR, MS/MS, HPLC, and chiral GC–MS analyses are preferred for structure evaluation [63][84]. Proton NMR (^{1}H NMR) spectroscopy has gained attention as a good analytical tool for structural analysis of polysaccharides (including determination of monosaccharide constituents, partial depolymerization by reductive hydrolysis, identification of disaccharide repeating units) and sequence analysis by enzymatic degradation due to its advantages of simple calibration, easy application, and fast optimization of the experiment [36][63][79]. However, this technique was only suggested for chemical identification and not quantification, due to possible structural irregularities, which could lead to misleading and complex signals. The linkage positions of carbohydrates and the linking relationships are determined concomitantly with heteronuclear single-quantum correlation spectroscopy (HSQC) and heteronuclear multiple bond correlation spectroscopy (HMBC) [36][84][86]. Globally, hydrocarbons characterization is mainly done by GC/MS and NMR [87].

Thermogravimetric analysis (TGA), differential scanning calorimetry (DSC), and dynamic mechanical analysis (DMA) were used to analyze thermal properties of polysaccharides, lipids (from supercritical extracts), and algal proteins, which can be quantified by determining the nitrogen content using Kjedhal analysis [36][86].

Infrared (IR) spectrometry is a common analysis technique used to identify functional groups present in algal extracts [79]. Thereby, glycoprotein structural details (e.g., sugars attached to the protein via (1→4)-linked β-galactose residues and β-linked glucose residues) have been elucidated using Fourier-transform infrared (FTIR) and NMR spectra [54][80]. Furthermore, glycoproteins obtained from *Codium decorticatum* were purified and characterized using HPLC, IR, NMR, and Circular Dichroism (CD) [15]. Generally, IR-KBr plate (mixing the powder sample with potassium bromide (KBr) and then pressing it into a disc mode) helped to identify algae's (e.g., Ulvan's) chemical components [36][86]. Further, attenuated total reflectance-FTIR (ATR-FTIR) and Raman spectroscopy techniques are used to identify agar and other polysaccharides sources of seaweeds [63]. Spirulina is an important edible alga with increasing commercial interest, and a faster and more highly efficient analytical platform was introduced to qualitatively and quantitatively characterize *Spirulina* pigments in different dietary supplements [87]. Thereby, analysis of the *Spirulina* pigment fraction was possible through a highly complex and developed analytical strategy, consisting of Fourier-transform ion cyclotron (FT–ICR) in both direct infusion (DIMS) mode or coupled with UHPLC. This strategy was used to accurately identify and overcome failures of conventional LC–MS-based methods (e.g., low separation efficiency, long analysis time, and low mass accuracy) [79][87].

TLC can be employed to elute extracts of chlorophyll α and multiple carotenoids, such as β-carotene, oscillaxanthin, zeaxanthin, β-cryptoxanthin, echinenone, and myxoxanthophyll [87]. The TLC method evaluates both quantitatively and qualitatively extracted algal components (e.g., hydrocarbons) among different solvents (mobile phases such as acetic acid/hexane/acetone/diethylamine/diethyl ether) and temperatures [36][87].

Several chromatographic methods, such as TLC, HPLC, GC, high-performance anion-exchange chromatography-pulsed amperometric detector (HPAEC–PAD), and CE, have been used for the separation and selective analysis of agaro-oligosaccharides (AOS) [35][84].

ESI and matrix-assisted laser desorption/ionization (MALDI) have advanced the structural analysis of AOS and carrageenan oligosaccharides (COS). Different fragmentation patterns were obtained by ESI-tandem MS due to sulfation substitution allowing researchers to selectively detect COS among other polysaccharides [35][63]. Thereby, detailed oligosaccharide information, such as accurate molecular weight, chain length distribution, fragments information, monosaccharide compositions, linkages, and location of various modifications, has been identified [35]. Recently, MS has been used as a powerful detection tool for elucidating the oligosaccharide structure due to its sensitivity [79][84].

For the quantitative analysis of toxins, LC–MS/MS methods have proven their efficiency, although they are limited for multi-component analyses (MCA) [36].

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